Rev. Écol. Biol. Sol, 1980, **17** (4) : 577-581.

Isolation and identification of bark decaying and utilizing bacteria of various origins

BY

A. M. DESCHAMPS, G. MAHOUDEAU, L. LEULLIETTE and J.-M. LEBEAULT

Division Procédés Biotechnologiques - Université de Technologie
B.P. 233 - 60206 Compiègne - France

INTRODUCTION

Recent investigations on wood and bark decay have proved that bacteria are probably as largely involved as fungi in the biodegradation of wood and bark components (GREAVES, 1971; ROSSELL *et al.*, 1973) but the bacterial contribution is not yet well described and understood. It is now sure that bacteria may degrade and assimilate not only cellulose but also different kinds of lignin as proved by the way of ^{14}C labelled lignins (HAIDER *et al.*, 1978) and the degradation of kraft-lignin (DESCHAMPS *et al.*, 1980 a), but also tannic acid and hydrolyzable tannins (DESCHAMPS *et al.*, 1980 b). But to our knowledge pure cultures made with bark or bark extract as the sole carbon source have never been reported for bacteria to prove that they are well adapted to decay and assimilate it. Same experiments have been reported for fungi only by UPDEGRAFF *et al.* (1975). So we investigated the possibility to isolate bacterial strains which could grow in these experimental conditions and succeeded in the isolation of 26 strains of bark decaying bacteria which could assimilate also one or many components of wood.

I. — MATERIALS AND METHODS

1. Cultures and isolation methods.

An enrichment culture technique was used to isolate bacterial strains able to grow with oak bark powder as the sole carbon source. A minimal medium previously descri-

Reçu le 6-5-80.

bed (DESCHAMPS *et al.*, 1980 a) was complemented with bark powder prepared by drying and milling oak bark samples to obtain approximately 1 mm size particles. The cultures were realised in 500 ml baffled flasks. The broth cultures were inoculated with various samples of soil, forest litter and decaying bark or wood (including samples of oak and pine). The first flask contained 2 g/l of glucose and the glucose was removed from the second flask on. The cultures were transfered every 5 days and the pH was adjusted at 7.0 to favorize the bacterial growth. The temperature was 37° C to obtain both the mesophilic strains and the thermophilic or thermotolerant ones. At the end enrichment culture series, dilutions were realised on Trypcase-Soy Agar (Difco) and the differents kinds of colonies observed were picked up and collected on Petri dishes. These strains were then tested separately as pure cultures in the same sterile medium used for the selection with bark powder as the sole carbon and energy source.

2. Characterization of the bacterial strains.

Among the positive strains isolated as described above, the capacities of some of them to degrade and assimilate complex carbon compounds considered as bark components were tested :

— Cellulolysis was detected with the cellulose-azure test based on the release of a dye compound by cellulolytic strains (DESCHAMPS and LEBEAULT, 1980 c).

— Lignin degradation was tested with reference to the assimilation of a commercial kraft-lignin Indulin AT (Westvaco Polychemicals, USA) which may be considered as a good lignin model for biodegradation tests (DESCHAMPS *et. al.*, 1980 a) as the sole carbon source.

— Xylan degradation was tested as the most largely distributed hemicellulosic compound in wood and bark samples, with xylan (Sigma) as the sole carbon source (DESCHAMPS and LEBEAULT, 1980 d).

— Assimilation of tannic acid as a model compound for hydrolyzable tannins was realised in broth cultures with tannic acid (Sigma) as the sole carbon source as tannins have been considered generally as bacteriostatic compounds and could prevent the bacterial growth in bark. But recent investigations have proved that bacteria may degrade and assimilate tannic acid and probably natural hydrolyzable tannins (DESCHAMPS *et al.*, 1980 b). The presence of tannins in the broth cultures were detected with a protein precipitation method (HAGERMANN and BUTLER, 1978).

— Pectinolysis was tested using 6 % and 14.5 % methylated pectins called respectively « Ruban rouge » and « Ruban brun » (kindly supplied by « Société Française Unipectine ») and the assimilation of Polygalacturonic Acid (Sigma) was also tested as the sole carbon source.

3. Identification of strains.

Routine bacteriological tests were used to give a presumptive identication of the bacterial strains with particular reference to the following tests: Gram stain, catalase, oxidase, fermentation of glucose, motility, reduction of nitrates, gelatinase, citrate degradation and acid production from different sugars. The Bergey's Manual of Determinative Bacteriology (BUCHANAN and GIBBONS, 1974) was followed for these identifications.

II. — RESULTS AND DISCUSSION

1. Isolation of strains.

Different series of flasks with various inocula were realised to obtain the largest amount of isolates. More than 200 colonies were primary isolated and tested as pure cultures but a large number of it failed to grow. A slight growth was considered as negative. A selection of 26 strains with a good positive growth was then realised.

When considering that the presence of about 0.5 g/l of tannins which could precipitate proteins was detected in the broth cultures, these isolates were probably tannin-resistant or tannin-degrading strains.

2. **Characterization of the nutrition capacities of the isolates and identification.**

Among the 26 strains, the distribution of the ability to assimilate different carbon components occurring in wood or bark was as following:

— 9 strains were cellulolytic and could grow easily on carboxymethylcellulose as the sol carbon and energy source and were positive rapidly when tested with the cellulose-azure test. These strains were distributed among 3 genera: 6 strains were identified as *Bacillus sp.*, 2 strains as *Cellulomonas* sp. and 1 strain as *Pseudomonas* sp.

— 12 strains were able to degrade and assimilate xylan: 5 strains were identified as *Bacillus* sp., 2 strains as *Aeromonas* sp., 1 strain as *Arthrobacter* sp., 1 strain as *Agrobacterium* sp., 1 strain as *Corynebacterium* sp., and 1 non-identifiable strain (Gram positive and fermentative large cells disposed as single, twice or tetrads, non-spore forming). As suggested in an other paper (DESCHAMPS and LEBEAULT, 1980 d) the occurrence of xylan degradation among wood decaying bacteria is largely distributed as for bark decaying bacteria.

— 3 strains were able to grow with kraft-lignin as a sole carbon source: one strain was identified as *Klebsiella pneumoniae*, 1 strain as *Aeromonas* sp. and 1 as *Pseudomonas* sp. We obtained also several strains of *Klebsiella* when screening for lignin degrading strains (DESCHAMPS *et al.*, 1980 a) and tannic acid degrading strains (DESCHAMPS *et al.* 1980 b). These results bring new evidences for the adaptation of such bacteria to botanical environments (KNITTEL *et al.*, 1977). A complete characterization of these different isolates of *K. pneumoniae* has been realised (manuscript in preparation). It must be noticed that some strains of *Pseudomonas* were also isolated from papermill effluents by MARCHAND (1978).

— Curiously the degradation of tannic acid was only observed for 3 strains: 2 strains of *Bacillus* sp. and one strain of *K. pneumoniae*. So the resistant strains seem to be the most widely distributed among these bark decaying isolates, when tannic acid and probably tannin-degrading bacteria seem to be not so frequent (DESCHAMPS *et al.*, 1980 b).

The tannin content of different cultures was directly determined and expressed as O.D. (optical density) and this content was almost the same in the different cultures and did not influenced directly the growth of the bacterial population.

— Pectinolysis was tested only for some strains: 2 strains of *Bacillus* sp., 1 strain of *K. pneumoniae*, 1 strain of *Aeromonas* sp. and one strain of *Arthrobacter* sp. These strains degraded 6 and 14,5 % methylated pectins and excepting *K. pneumoniae* degraded polygalacturonic acid.

III. — CONCLUSION

These results bring new evidence for the bacterial part in the biodegradation of bark in situ or in the soil. Several species of active degrading bacteria have been isolated and grew on bark powder without adding any additional carbon source. Till now bacteria have been generally considered as secondary microorganisms in wood decay and secondary colonizers that could only assimilate the degradation products from the fungal population (BLANCHETTE and SHAW, 1978). This and the other papers we published oblige us to modify our opinion and conclude that bacteria are also active degraders, but it seems that they are not so numerous than fungi but also good degraders. The fact that bacteria may degrade and assimilate tannic acid and hydrolyzable tannins (DESCHAMPS *et al.*, 1980 b ; unpublished results) but also lignin and xylan are of primary importance. Some strains have been noticed to degrade and assimilate several compounds simultaneously as Aeromonas sp. and K. pneumoniae.

Investigations are now centered on the potential utilizations of selected bacterial strains to manage or improve bark decay to obtain a compost-like product or to eliminate rapidly forest residues directly or in the soil.

SUMMARY

Enrichment cultures were realised with oak milled bark powder as the sole carbon and energy source to screen for the bacterial population obtained from soil, forest litter and decaying wood and bark samples. Several types of bacterial isolates were then obtained but 26 strains on more than 200 strains tested were able to grow as pure cultures with bark powder. Among the isolates 9 cellulolytic strains, 12 xylan degrading strains, 3 lignolytic strains, 3 tannin degrading strains and 5 pectinolytic strains were identified. These results bring new evidence for the active bacterial part in wood and bark decay « in situ » or in the soil.

RÉSUMÉ

Des cultures par enrichissement ont été faites à partir d'échantillons de sol, de terreau et de bois et d'écorce en décomposition, avec de l'écorce de chêne broyée comme seule source de carbone. Un grand nombre de types bactériens ont pu être isolés à partir de ces cultures mais seules 26 souches se sont montrées capables de se développer en cultures pures avec de l'écorce. Parmi celles-ci 9 souches étaient cellulolytiques, 12 dégradaient le xylane, 3 souches dégradaient la lignite kraft, 3 dégradaient l'acide tannique et 5 étaient pectinolytiques. Ces résultats montrent le rôle actif et jusqu'ici sous estimé joué par les bactéries dans la biodégradation du bois et de l'écorce « in situ » et dans le sol.

REFERENCES

BLANCHETTE (R. A.) and SHAW (C. G.), 1978. — Associations among bacteria, yeasts and basidiomycetes during wood decay. *Phytopathol.*, **68**: 631-637.

BUCHANAN (R. E.) and GIBBONS (N. E.) eds, 1972. — Bergey's Manual of Determinative Bacteriology. 8th ed. The Williams and Wilkins Co., Baltimore.

DESCHAMPS (A. M.), MAHOUDEAU (G.) and LEBEAULT (J. M.), 1980 a. — Fast degradation of kraft lignin by bacteria. *Eur. J. Appl. Microbiol. Biotechnol.*, **9**: 45-51.

DESCHAMPS (A. M.), MAHOUDEAU (G.), CONTI (M.) and LEBEAULT (J. M.), 1980 b. — Bacteria degrading tannic acid and related compounds. *J. Ferment. Technol.*, **58**: in press.

DESCHAMPS (A. M.) and LEBEAULT (J. M.), 1980 c. — Recherche de bactéries cellulolypiques par la méthode à la cellulose-azure. *Ann. Microbiol.* (Inst. Pasteur), **131 A**: 77-81.

DESCHAMPS (A. M.) and LEBEAULT (J. M.), 1980 d. — A survey of xylan degradation among wood decaying bacterial isolates. *Eur. J. For. Path.*, **10**: in press.

HAGERMANN (A. E.) and BUTLER (L. G.), 1978. — Protein precipitation method for the quantitative determination of tannins. *J. Agric. Food Chem.*, **26**: 809-812.

GREAVES (H.), 1971. — The bacterial factor in wood decay. *Wood Sci. Technol.*, **5**: 6-16.

HAIDER (K.), TROJANOWSKI (J.) and SUNDMAN (V.), 1978. — Screening for lignin degrading bacteria by means of ^{14}C labelled lignins. *Arch. Microbiol.*, **119**: 103-106.

MARCHAND (M.), 1978. — Activité lignolytique de quelques microorganismes isolés d'une station d'épuration d'effluents de papeterie. *Rev. Ecol. Biol. Sol*, **15**: 323-331.

ROSSELL (S. E.), ABBOT (G. M.) and LEVY (J. F.), 1973. — Bacteria and wood, a review of the literature relating to the presence, action and interaction of bacteria and wood. *J. Inst. Wood Sci.*, **6**: 28-35.

UPDEGRAFF (D. M.) and GRANT (W. D.), 1975. — Microbial utilization of Pinus radiata bark. *Appl. Microbiol.*, **30**: 722-726.